Remarks

Claims 42, 52-59, 61, 62, 64-67, and 69-81 were pending in the subject application. By this Amendment, claims 42, 57, 59, 66, 67, 69, 71, 72, 74, 77, and 79 have been amended, claims 53-55, 58, 61, 62, 70, and 76 have been cancelled, and new claims 82 and 83 have been added. The undersigned avers that no new matter is introduced by this Amendment. Support for the new claims and amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 42, 52, 56, 57, 59, 64-67, 69, 71-75, and 77-83 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

Applicants and Applicants' representative wish to thank Examiner Schnizer for the courtesy of the telephonic interview conducted with the undersigned on July 19, 2010 regarding the Raviprakash *et al.* publication, which is cited in the Office Action, and as it relates to claims 59, 71, and 77 of the pending claims, which recite a target sequence common to four DV serotypes.

Submitted herewith is a Request for Continued Examination (RCE) under 37 CFR §1.114 for the subject application.

Submitted herewith is a supplemental Information Disclosure Statement (IDS), accompanied by the form PTO/SB/08 and copies of the references listed therein. Applicants respectfully request that the references listed on the form PTO/SB/08 be considered and made of record in the subject application.

By this Amendment, claims 42, 57, 59, 66, 67, 69, 71, 72, 74, 77, and 79 have been amended and claims 82 and 83 have been added. Support for the amendments to claims 42 and 72 can be found, for example, at page 12, lines 3-24; page 14, lines 5-25; page 15, lines 1-9; and the Examples at pages 25-34 of the specification as filed. Support for the amendment to claim 57 can be found, for example, at page 24, lines 1-2, of the specification. Support for the amendments to claims 59, 71, and 77 can be found, for example, at page 10, lines 9-10, and page 28, lines 25-28, of the specification. Support for the amendments to claims 66, 67, 74, and 79 can be found, for example, at page 11, lines 26-28, of the specification. Support for the amendment to claim 69 can be found, for example, at page 7, lines 19-20; page 8, lines 3-5, and the Examples of the specification. Support for

claim 82 can be found, for example, at page 10, lines 9-10, and page 28, lines 25-28, of the specification. Support for claim 83 can be found at page 24, lines 1-2, of the specification.

Claims 42, 52-55, 58, 59, 64, 69, 71-73, 75-77, 79, and 81 are rejected under 35 USC §103(a) as obvious over Iversen *et al.* (U.S. Published Application 2005/0096291), Raviprakash *et al.* (*J Virol,* 1995, 69(1):69-74), Adelman *et al.* (*J Virol,* 2002, 76(24):12925-12933), Tuschl *et al.* (U.S. Patent 7,056,704), and Yu *et al.* (*PNAS*, 2002, 99(9):6047-6052). Claim 57 is rejected under 35 USC §103(a) as obvious over Iversen *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.* (*PNAS*, 2002), as applied to claims 42, 52-55, 58, 59, 64, 69, 71-73, 75-77, 79, and 81 above, and further in view of Yu *et al.* (U.S. Patent 6,852,528). Claims 61 and 62 are rejected under 35 USC §103(a) as obvious over Iversen *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.* (*PNAS*, 2002), as applied to claims 42, 52-55, 58, 59, 64, 69, 71-73, 75-77, 79, and 81 above, and further in view of Kumar *et al.* (U.S. Patent 7,067,633). Claims 65-67 and 70 are rejected under 35 USC §103(a) as obvious over Iversen *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.* (*PNAS*, 2002), as applied to claims 42, 52-55, 58, 59, 64, 69, 71-73, 75-77, 79, and 81 above, and further in view of Hope *et al.* (U.S. Patent 6,136,597). Applicants respectfully assert that the claimed invention is not obvious over the cited references and traverse the rejections of record.

Applicants note that each of the aforementioned rejections under 35 USC §103(a) rely on the Iversen *et al.* patent, and the earliest claimed priority date of the Iverson *et al.* patent is August 5, 2003. Claims 42, 52, 56, 57, 59, 64-67, 69, 71, and 80 as amended are entitled to the filing date of provisional application number 60/319,964, filed February 21, 2003, and/or provisional application number 60/320,108, filed April 15, 2003, which precede the earliest claimed priority date of the Iversen *et al.* patent. Support for claim 42 and 64-67 can be found, for example, in paragraph [0002] at page 1; paragraphs [0010] – [0019] at pages 4-6; and the figures, abstract and claims of provisional application number 60/319,964; and in paragraph [0001], at page 1; paragraphs [0006] – [0008] at page 6-7, and the figures, abstract, and claims of provisional application number 60/320,108. Support for 56 can be found in Figure 2B at page 19 of provisional application number 60/320,108. Support for claim 57 can be found in claim 3 of provisional application number 60/320,108. Support for claims 59 and 71 can be found, for example, in paragraph [0021] at page 12 of provisional application number 60/320,108. Support for claim 69 can be found, for example, in

Example 3 at page 13, and the abstract and claims of provisional application number 60/320,108. As the earliest claimed priority date of the Iverson *et al.* patent is after the effective filing date of the subject application, the Iverson *et al.* patent is not available as prior art under 35 USC §103(a).

At page 4, the Office Action indicates that "it would have been obvious to target any of the DV genomic RNA regions set forth by Iversen *et al.*, Raviprakash *et al.*, or Adelman *et al.* because the genome of DV is a positive strand RNA encoding a single polyprotein, such that cleavage of any of the targets would have been expected to inhibit DV replication." Included with the supplemental Information Disclosure Statement (IDS) submitted herewith is the Nawtaisong *et al.* publication (*Virology Journal*, 2009, 6:73), which was published more than five years after the application's filing date and states that size restrictions of target sequences limits the number of targets that are conserved among all DV strains, and escape mutants can result from a single point mutation among the target sequence (see Background section). This is particularly relevant with respect to claims 59, 71, 77, and 82. Furthermore, it was known that at least some flaviviruses seem to evade the RNAi response by sequestering their replication complex inside a double-layered membrane complex. Nawtaisong *et al.* further teach that another potential obstacle in using RNAi is the evidence for viral RNAi suppressors which have been reported in plants, poliovirus, and HIV-1 variants.

At page 4, the Office Action indicates that paragraph [0071] of the Iversen *et al.* publication taught that treatment could be either prophylactic or post-infection. As indicated above, the effective filing date of at least some of the currently pending claims predates that of the Iverson *et al.* patent and, therefore, the Iverson *et al.* patent is not prior art to those claims. Furthermore, this mere statement in the Iverson *et al.* patent provides no reasonable expectation of success in achieving the effects recited in the pending claims before or after DV infection.

The Subramanya *et al.* publication (*Journal of Virology*, Mar. 2010, 84(5):2490-2501), of record, supports the non-obviousness of the claimed invention, and was published more than five years after the application's filing date. As described in Subramanya *et al.*, dendritic cells are of special relevance to dengue virus infection, and a hurdle for RNAi therapeutics is "the specific delivery of small interfering RNA (siRNA) to relevant cell types" (page 2491, left column, first full sentence):

> "Dengue-infected DCs play a key role in the immunopathogenesis of DHF/DSS, as, along with macrophages, they release proinflammatory cytokines and soluble factors that mediate plasma leakage, thrombocytopenia, and hypovolemic shock associated with severe dengue infection (14, 15, 29, 38). Therefore, development of a method to introduce siRNA into DCs would be an important step toward using RNAi therapeutically to suppress viral replication and/or to attenuate the vigorous host cytokine responses in dengue infection (7, 19)" (page 2491, left column, first full paragraph, of Subramanya *et al.*, emphasis added).
>
> "DCs are of special relevance to dengue infection, as they are the initial cells in the skin to become infected during transmission of the virus by infected mosquito bite (12), and the proinflammatory cytokines that they produce play a significant role in dengue immunopathogenesis (13, 14, 38)" (page 2497, left column, last paragraph, of Subramanya *et al.*).

This important step of developing a method to introduce dengue virus-targeted siRNA into dendritic cells (DCs) to inhibit viral replication was first achieved by the inventors of the subject invention. Indeed, Subramanya *et al.* indicate that nonselective methods have been used successfully for *in vivo* delivery of siRNA to liver and other tissues; "however, they may not work well for primary hematopoietic cells such as DC" (page 2498, left column, of Subramanya *et al.*, emphasis added). As described in Examples 8 and 9 of the subject application, the inventors of the subject invention determined that interfering RNA targeting dengue virus can effectively be delivered to human dendritic cells, decrease dengue virus infection, and inhibit dengue virus-induced apoptosis of these cells. In contrast, the primary reference relied upon in each of the rejections under 35 USC §103(a), the Iversen *et al.* publication, describes an experiment in which antisense oligonucleotides inhibited replication in Vero cells, which are kidney epithelial cells of the African Green Monkey (see Example 3, at page 15, paragraphs [0179] and [0180] of the Iversen *et al.* publication). Uptake of the antisense oligonucleotides by dendritic cells and inhibition of dengue virus infection of dendritic cells was not evaluated in the Iversen *et al.* publication.

At page 13, the Office Action states that "Applicant has presented no clear evidence that one of ordinary skill would have doubted that at least some DCs would have been transfected by the methods of the combined references, and that DV gene expression would have been inhibited in those transfected cells" (emphasis added). The claims as amended recite more than transfection of some DCs. For example, independent claim 42 as amended recites a method for attenuating DV

infection in human cells susceptible to DV infection *in vivo*, and administration of an effective amount of the vector. Claim 69 recites that the siRNA molecule attenuates DV replication in the cells. Independent claim 72 recites a method for inhibiting DV infection and DV-induced apoptosis of human dendritic cells *in vivo*, and administration of an effective amount of the vector. Claim 73 recites that the cells are subsequently exposed to DV, and the siRNA molecule inhibits DV infection and DV-induced apoptosis in the cells. In rejecting claims as *prima facie* obvious under 35 USC §103(a), it is the burden of the Patent Office to establish that the teachings of the prior art provide a sufficient basis for a reasonable expectation of success.

At pages 13-14, the Office Action cites the Palmowski *et al.* (*J. Immuno.*, 2004, 172:1582-1587), Condon *et al.* (*Nature Medicine*, 1996, 2(10):1122-1128), Song *et al.* (*Proc. Nat. Acad. Sci. USA*, 1997, 94:1943-1948), Porgador *et al.* (*J. Exp. Med.*, 1998, 188(6):1075-1082), Barratt-Boyes *et al.* (*J. Immunol.*, 2000, 164:2487-2495), and Larregina *et al.* (*Gene Therapy*, 2001, 8:608-617) publications to show that one of ordinary skill in the art at the time of the invention would have been aware that DCs could have been transfected with expression vectors *in vivo*. However, Applicants note that none of these references appear to demonstrate the successful targeting of endogenous exogenous viral genes within DCs using RNA interference.

In the Raviprakash *et al.* publication, modified phosphorothioate antisense oligonucleotides and unmodified antisense oligonucleotides were injected into LLCMK/2 cells, which are cells of a rhesus monkey kidney cell line, not dendritic cells. Furthermore, the results of the Raviprakash *et al.* publication, independently or in combination with the other cited references, would not have led one of ordinary skill in the art to the claimed methods with any reasonable expectation of success. The Raviprakash *et al.* publication discloses that unmodified antisense oligonucleotides were not effective in bringing about significant inhibition of DV (see abstract), and that the 3'a antisense oligonucleotide, which targeted a sequence in the 3' untranslated region (UTR) shared by all four DV serotypes, showed "limited efficacy." Raviprakash *et al.* attributed this result to the complex secondary structures presented by the large (>10 kb) DV RNA (page 74, first full paragraph). In contrast to these results with 3' UTR-targeted antisense oligonucleotides, Applicants note that the subject specification demonstrates very effective inhibition of DV infection and DV-induced apoptosis in human dendritic cells. Finally, the Raviprakash *et al.* publication concludes that the

modified phosphorothioate oligonucleotides may be generally more effective as antisense agents against other viruses (page 74, last sentence).

Applicants respectfully submit that, at the time the subject application was filed, even successful targeting of specific regions with antisense oligonucleotides did not necessarily confer a reasonable expectation of success in those regions with interfering RNA. Again, in Raviprakash *et al.*, the unmodified oligonucleotides were not effective. Accessibility of target regions by antisense oligonucleotides did not necessarily confer a reasonable expectation of success by the effector complex (the RNA-induced silencing complex (RISC)) in RNA interference.

The Adelman *et al.* publication describes intrathoracic injection of mosquitos (*Aedes aegypti*) with vectors encoding sense and anti-sense RNA-mediated interference molecules. While the Adelman *et al.* publication suggests new ways of inhibiting replication of dengue virus in mosquito vectors, it is not relevant to inhibition of dengue virus replication in human cells or a human.

The Yu *et al.* publication is cited for teaching RNA interference by expression of hairpin siRNAs and their use in mammalian cells. The Office Action concludes that one skilled in the art would have been motivated to substitute the antisense oligonucleotides of the Iversen *et al.* publication because the Tuschl *et al.* publication taught that siRNAs were more efficient than antisense. The Yu *et al.* and Tuschl *et al.* publications do not address the aforementioned deficiencies of the Iversen *et al.*, Raviprakash *et al.*, and Adelman *et al.* references.

Furthermore, as indicated by Subramanya *et al.*, dengue pathogenesis is characterized by overproduction of proinflammatory cytokines, including TNF-alpha, which is implicated in the vascular leakage that characterizes DHF/DSS and the plasma levels of which are elevated during acute dengue infection (see page 2498, right column, of Subramanya *et al.*). Therefore, an important limitation to be considered is whether blockade of host molecules such as TNF-alpha "also interferes with a possible antiviral effect that might outweigh its pathogenic potential." Not only did the inventors of the subject invention empirically determine that interfering RNA could be successfully delivered to human dendritic cells, decrease dengue virus infection, and inhibit dengue virus-induced apoptosis of human dendritic cells, the inventors determined that the interfering RNA did not induce acute inflammation in human dendritic cells as determined by the level of pro-inflammatory

cytokines, including TNF-alpha, as shown in Figure 10 and described in Example 10 at page 31 of the subject specification.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art. MPEP §2143.01. Obviousness does not require absolute predictability, however, at least some degree of predictability is required. MPEP §2143.02. Evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness. *In re Rinehart*, 531 F.2d 1048, 189 USPQ 143 (CCPA 1976). Furthermore, assuming *arguendo* that it would have been obvious to try administering the claimed vector to human cells *in vivo* to attenuate DV infection, it is well established that obvious to try is an acceptable rationale in support of a conclusion of obviousness when choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success. MPEP §2141. Such is not the case here, for the reasons cited above.

Claim 57 is rejected over Iversen *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.*, as applied to claims 42, 52-55, 58, 59, 64, 69, 71-73, and 75-77 above, and further in view of Yu *et al.* (U.S. Patent 6,852,528). The Yu *et al.* patent is relied upon for teaching a variety of methods to deliver nucleic acids to cells. The deficiencies of the other references are described above. The Yu *et al.* patent does not cure those deficiencies or confer a reasonable expectation of success in administering the claimed vector to human cells susceptible to DV infection *in vivo* or human dendritic cells *in vivo*, as recited in the currently pending claims.

Claims 61 and 62 are rejected over Iversen *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.*, as applied to claims 42, 52-55, 58, 59, 64, 69, 71-73, and 75-77 above, and further in view of Kumar *et al.* (U.S. Patent 7,067,633). The Kumar *et al.* patent is relied upon in the Office Action for teaching the importance of promoters and/or enhancers to direct expression of a DNA segment. The deficiencies of the other references are described above. The Kumar *et al.* patent does not cure those deficiencies or confer a reasonable expectation of success in administering the claimed vector to to human cells susceptible to DV infection *in vivo* or human dendritic cells *in vivo*, in accordance with the currently pending claims.

Claims 65-67 and 70 are rejected over Iversen *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.*, as applied to claims 42, 52-55, 58, 59, 64, 69, 71-73, and 75-77 above,

and further in view of Hope *et al.* (U.S. Patent 6,136,597). The Hope *et al.* patent is relied upon in the Office Action for teaching that expression cassettes could be delivered by a varety of viral or non-viral vectors, including adeno-associated virus. The deficiencies of the other references are described above. The Hope *et al.* patent does not cure those deficiencies or confer a reasonable expectation of success in administering the claimed vector to human cells susceptible to DV infection *in vivo* or human dendritic cells *in vivo*.

Applicants respectfully submit that the claimed invention is not obvious over the cited references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

Claims 72, 73, 75, and 77 are rejected under 35 USC §103(a) as obvious over Libraty *et al.* (*J Virol,* 2001, 75(8):3501-3508), Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.* (*PNAS*, 2002). Claim 74 is rejected under 35 USC §103(a) as obvious over Libraty *et al.*, Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, and Yu *et al.* (*PNAS*, 2002) as applied to claims 72, 73, 75, and 77 above, in further view of Hope *et al.* Applicants respectfully assert that the claimed invention is not obvious over the cited references and traverse the rejections of record.

The Office Action indicates that Libraty *et al.* disclose dendritic cells infected by DV and indicate that these cells were relevant to understanding the pathogenesis of DV and the development of therapeutic strategies. Furthermore, the Office Action indicates that it would have been obvious to one of ordinary skill in the art at the time of the invention to use the cells of Libraty *et al.* in the experiments of Raviprakash *et al.* Applicants agree that the Libraty *et al.* publication disclose dendritic cells infected by dengue virus, and that these cells are relevant to understanding the pathogenesis of dengue virus and the development of therapeutic strategies. Applicants' remarks in response to the aforementioned rejections based on the other cited references (Raviprakash *et al.*, Adelman *et al.*, Tuschl *et al.*, Yu *et al.*, and Hope *et al.*) are applicable here, and those remarks are incorporated herein by reference. The cited references do not confer one of ordinary skill in the art with any reasonable expectation of success in carrying out the claimed methods at the time of the invention for the reasons stated above. Applicants respectfully submit that the claimed invention is not obvious over the cited references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

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